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Localised epidermal drug delivery induced by supramolecular solvent structuring

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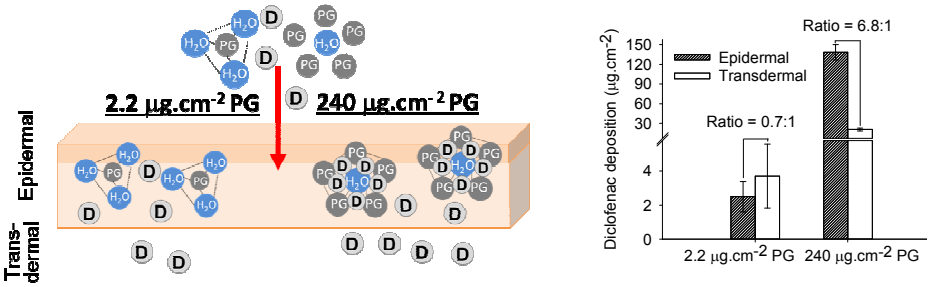
Graphical Abstract



Forming propylene glycol (PG) rich solvent supramolecular structures in human skin enhances the epidermal localisation of diclofenac (D) upon topical administration.

Abstract

The preferential localisation of drug molecules in the epidermis of human skin is considered advantageous for a number of agents, but achieving such a delivery profile can be problematic. The aim of the present study was to assess if the manipulation of solvent supramolecular structuring in the skin could be used to promote drug residence in the epidermal tissue. Skin deposition studies showed that a 175-fold increase in the epidermal loading of a model drug diclofenac (138.65 ± 11.67 $\mu g.cm^{-2}$), compared a control (0.81 ± 0.13 $\mu g.cm^{-2}$), could be achieved by co-localising the drug with a high concentration of propylene glycol (PG) in the tissue. For such a system at 1 h post-dose application the PG flux was into the skin was 9.3 $mg.cm^{2}.h^{-1}$ and the PG:water ratio in the epidermis was 76:24 (v/v). At this solvent ratio infrared spectroscopy indicated that PG rich supramolecular structures, which displayed a relatively strong physical affinity for the drug, were formed. Encouraging the production of the PG-rich supermolecular structures in the epidermis by applying diclofenac to the skin using a high PG loading dose (a 240 $\mu g.cm^{-2}$) produced an epidermal:transdermal drug distribution of 6.8:1. However, generating water-rich solvent supermolecular structures in the epidermis by applying diclofenac using a low PG loading dose (2.2 $\mu g.cm^{-2}$), led to a loss of preferential epidermal localisation of diclofenac in the tissue (0.7:1 epidermal:transdermal drug distribution). This change in diclofenac skin deposition profile in response to PG variations and the accompanying FTIR data supported the notion that supramolecular solvent structures could control drug accumulation in the human epidermis.

Keywords: Human skin; topical delivery; supramolecular structuring; propylene glycol; diclofenac.

Introduction

The human skin is a highly stratified organ. Its different layers show diversity in their composition. For example, the water content in the outermost layer, the *stratum corneum,* is ca. 20% w/w, in the underlying viable epidermis it is 60% w/w whilst in the dermis, which is innovated by blood, it is > 70% w/w [1, 2]. When drugs are applied topically to the skin the different characteristics of the layers dictate the regions in which the administered agents will preferentially reside. The distribution of a drug in the skin can be modified by altering the contents of important components already in the skin, e.g., Solaraze®, which is thought to modify skin hydration, or by delivering molecules into the tissue which modify its properties [3-5]. Research has shown that the co-localisation of drugs with excipients in the skin is one of the most effective means to alter the distribution of the agents in the different skin strata but, the drug-excipient interactions that occur in the skin are not yet so well understood that topical formulations can be engineered to concentrate a drug in specific strata of the skin and this suggests that more work is needed in this field [6-10].

It is notable that some of formulation excipients that have been reported to readily pass into the skin are also known to also display non-ideal mixing characteristics when combined with water (e.g. ethanol [11], propylene glycol (PG) [12-13] and polyethylene glycol [14]). This behaviour is a consequence of supramolecular structure formation by the molecules in an aqueous fluid. However, there have been no previous reports that document how solvent structuring influences the manner in which a co-administered drug distributes in skin tissue. It is possible that manipulating the formation of solvent supramolecular structures could act as a novel means to achieve epidermal localisation of an active therapeutic agent. However, this would require the level of the excipient to be controlled such that physical interactions that occur

between the supramolecular structures and the drug promote its retention within a particular layer of the tissue. To evaluate this potential mechanism of skin penetration enhancement more research is required to establish the nature of the interactions between topically applied drugs and co-localised excipients.

Solvent supramolecular structures do not form covalent bonds with therapeutic agents and facilitate their transport as a drug solvent complex, rather they penetrate into barriers and co-localise with drug molecules. Once in the tissue they are thought to modify the drug-tissue association by the competitive formation of drug-solvent bonds that occur through a range of temporal physical interactions; which include hydrophobic, electrostatic and hydrogen bond formation [15-17]. Drug-solvent and drug-tissue interactions form an equilibrium that is highly dynamic and dependant on the solvent-solvent structuring [18,19]. For non-ideally mixed systems these solvent-solvent structures can be very diverse in nature and can change depending upon the exact ration of molecules present in the solvent. For example, Dixt *et al.,* has reported that non-ideally mixed clusters of water and methanol could produce water structures of between 2-20 molecules in a methanol-rich mixture [16] and methanol clusters of between 3-8 molecules in a water-rich solvent mixture [15]. Whilst the exact size of these clusters have yet to be measured from their combined molecular mass it is evident that they could provide a complex with suitable properties that once generated within the skin strata might bind small molecular weight drugs in order to encourage co-localisation.

Recent studies that have shown PG-water solvent structuring can be used to manipulate drug transport through confluent barriers [12]. This work demonstrated that propylene glycol (PG), a diol widely used in topical formulations and known to form solvent supramolecular structures with water [12-13], acted by non-covalent binding to the drug within the barrier after topical

administration. The aim of the present study was to investigate whether through the control of PG solvent supramolecular structuring in the applied dosage form and in the skin the distribution of topically applied drugs within the human skin could be manipulated. A dual pump spray, described in a previous study [20], which allowed a drug to be delivered at a high level of drug saturation in a PG-water cosolvent, was utilised to deliver a model drug to the surface of the skin. Diclofenac was selected as the model agent as it was known to pass through the skin and its permeation was known to be influenced by PG [12]. An *ex vivo* human tissue model was used to test the percutaneous penetration of both the PG solvent and drug into the skin as it is thought to provide a reliable means to model topical drug delivery in man. As previous studies have shown that it is extremely problematic to measure the drug-solvent interactions once molecules have passed into the skin [21], in this work the rate of PG transport through the skin was measured and combined with the known rate of water transport in order to calculate the co-solvent ratios in the different strata of the tissue. This information was used to determine the levels of diclofenac and PG in the each strata of the tissue and enabled the final experimental design to be refined. Accordingly the PG loading on the surface of the skin was altered, in order to assess whether the solvent supramolecular structuring would modify the diclofenac disposition in the human epidermis.

Experimental Section

Materials

Diclofenac, as the diethylamine salt (melting point of 154°C, BP grade, 99.9%) was kindly donated by Unique Chemicals, India. HPMC (Metolose, grade 65SH, viscosity 50 cP), was a gift from Shin-Etsu Chemical Ltd, Japan. Propylene glycol (PG) (ACS reagent grade, ≥99.5%) was supplied by Sigma Aldrich, UK. Acetonitrile and methanol (high performance liquid chromatography (HPLC) grade) were obtained from Fisher Scientific International, UK and ethanol (99.7–100% v/v) was provided by BDH Laboratory Supplies, UK. Phosphate buffered saline (PBS, pH 7.2, 0.172 M) tablets were supplied by Oxoid Ltd, UK. Sheets of silicone membrane (Folioxane®), with a thickness of 120 μm, were purchased from Novatech Ltd, France. De-ionised water (electrical conductivity 0.5-1 μS) was used throughout this study.

Formulation preparation

The diclofenac-saturated PG solution was produced by adding an excess of the diclofenac diethylamine salt (DDEA) to PG, adjusting to pH 3 ± 0.1 with phosphoric acid and continually stirring at 25˚C, for 72 h (> 48 h was required to ensure equilibrium solubility was attained in PG; the kinetic solubility data to confirm the system had reached equilibrium was recorded but is not shown). The DDEA suspension was then centrifuged for 20 min at 16,060 g (Biofuge pico, Heraeus, Kendro Laboratory Products plc, UK) and the drug-saturated supernatant removed and used in the work. The final pH of all the drug-saturated solutions used in the study was checked and recorded (pH 3 ± 0.1).

The drug-supersaturated PG-water vehicle containing diclofenac microparticles (InDicP) was generated using a dual-canister pump spray (100 μL metered nozzle), described previously [20]. One canister contained diclofenac-saturated PG and the second contained HPMC (3 % w/v) dispersed in an aqueous buffer (PBS, 0.172 M, pH 3). To form the InDicP microparticulate formulation *in situ*, two sprays were simultaneous actuated and allowed to mix spontaneously on the surface of the skin to achieve a vehicle composition of 50:50 (v/v) PG:water. A comparison of diclofenac permeation across silicone membrane obtained after application using the two pump sprays with that recorded after administration of the two solutions using a pipette showed no significant difference and this confirmed effective dosing and mixing by the pump sprays (data not shown). The applied InDicP formulation contained diclofenac at 0.42% w/v and HPMC at 1.5% w/v.

Formulation characteristics

Infrared spectroscopy

The molecular interactions that occurred in the pure PG solvent, the pH 3 adjusted water and the 50:50 (v/v) PG-water solution were assessed using a demountable universal liquid transmission cell system (Omni-Cell, Specac Ltd., UK) fitted with CaF_2 windows and a 25 μm mylar spacer (Specac Ltd., UK) on a spectrum One Fourier transform infrared (FTIR) spectrometer (Perkin Elmer Ltd., UK). Deuterated water (D_2O) was employed to allow visualisation of PG absorption bands in the 1700-1300 cm^{-1} range. All the systems were carefully adjusted to a pH of 3.0 ± 0.1 to control the drug's ionisation. The drug (0.42% w/v) and HPMC (1.5% w/v) interactions with the PG, water and the 50:50 (v/v) mixture was assessed by solubilising the components in the vehicles and again carefully re-adjusting the

pH to 3.0 ± 0.1 prior to analysis. The InDicP was analysed directly upon formation and then repeatedly over a 180 min time course. All spectra were produced using 32 scans collected at a spectral resolution of 4 cm^{-1} using the Spectrum One software (version 10, Perkin Elmer Ltd., UK).

Diclofenac microparticle morphology

The morphology of the *in situ* assembled diclofenac microparticles (InDiP) was assessed in the solution state and compared to a control (equivalent system but in the absence of HPMC) using an optical microscope (Olympus BX50F, Japan) at a magnification of ×200.

Diclofenac saturation

The degree of diclofenac saturation in the vehicle of the InDicP formulation was determined through the assessment of the drug transmembrane transport and then relating this value to a flux-DS calibration curve derived using a 50:50 (v/v) PG:water vehicle (pH 3, containing 1.5% w/v HPMC), as reported previously [22]. Diclofenac transmembrane transport was assessed *in vitro* using individually calibrated upright Franz diffusion cells (MedPharm Ltd, UK) (diffusional area of ~2.1 cm^2 and receptor volume of ~10.8 ml) fitted with disks of silicone membrane (measured thickness of 124.17 ± 6.01 μm, n = 54) and containing a magnetic stirrer in the receiver compartment. The receptor compartment was filled with a previously sonicated and filtered receiver phase consisting of 20:80 (v/v) EtOH:PBS (pH 7.4). The assembled Franz cells were placed into a Variomag® magnetic stirrer plate immersed in a water bath (Grant Instruments, UK) and the study was initiated by the application of 10 actuations from the dual spray formulation which generated 1 ml of the

InDicP system on the apical surface of the silicone membrane. Aliquots of the receiver fluid (1 mL) were removed periodically and replaced immediately with the same volume of fresh receiver fluid, in order to monitor the diclofenac membrane permeation. The removed samples were analysed immediately for diclofenac content by HPLC. All the transport experiments were repeated 5 times and all the data were used.

Ex vivo human skin deposition studies

Surgically excised samples of human skin were obtained directly after abdominoplastic surgery with informed patient consent and approval from the School of Life Sciences Ethics Committee with Delegated Authority, University of Hertfordshire (Reference number: 07/H0711/72, Protocol LS12/11/06S). Epidermal sheets were prepared using the heat separation method [23]. The prepared epidermal sheets (ES) were blotted dry with tissue paper and stored flat, wrapped in aluminium foil at -20°C until use. Diclofenac and propylene glycol skin deposition was assessed using individually calibrated Franz-type diffusion cells (MedPharm Ltd., Guildford, UK). The 240 $\mu g.cm^{-2}$ PG dose studies required the application of 1 ml of InDicP. Initially the 240 $\mu g.cm^{-2}$ PG dose was compared to a saturated control system, Dic, with an equivalent PG loading, equivalent co-solvent composition (50:50 % (v/v) PG:water) and HPMC (1.5% w/v) levels, but with DS = 1 using a pre-formed homogenous solution of the drug and the excipients. The skin penetration of the diclofenac from the two formulations was measured over a time course of 24 h by taking a number of samples from the receiver solution of Franz diffusion cells which contained epidermal skin in order to establish the permeation profile. Subsequent comparisons were made of diclofenac diffusion following the application of a (high) 240 $\mu g.cm^{-2}$ PG dose with that attained after administration of a (low) 2.2 $\mu g.cm^{-2}$ PG loading dose. The latter required the application of

2.5 μL (3.3 μg.cm^{-2}) of the InDicP formulation, and only the amount of drug diffused after 24 h was determined, so as to determine the deposition of drug after that time period (including the amount of drug retained in the skin) following a single topical dose. In all the studies the Franz diffusion cells were fitted with human ES (two pieces of human abdominal skin were used; one for the low PG loading dose and another for the high PG loading dose and saturated drug control). Sonicated PBS (0.172 M, pH 7.4) was used as the receiver fluid. The ES was maintained at 32°C by immersing the Franz cells in a water bath (Grant Instruments, UK) held at 37°C. The donor compartment was covered with parafilm to control water loss during the duration of the studies. For the 240 μg.cm^{-2} PG loading dose and saturated control time-profile studies, 1 mL aliquots of the receiver fluid were removed at defined time points over a 24 h time course (this was extended to 26 h due to technical demands of the experiment) and replaced immediately with the same volume of fresh receiver fluid, in order to monitor the diclofenac and PG penetration. The removed samples were divided to analyse diclofenac (0.5 ml) and PG (0.5 ml) content as described below. At the end of the skin permeation studies, the amount of diclofenac localised in the ES was determined. Each Franz cell was dissembled and the donor compartment was swabbed with wet then dry cotton buds (4 in total) and two tape strips were used (Henkel consumer adhesives, UK), if required, to ensure the surface of the skin was free from the applied formulation (pilot studies showed significant drug remained on the surface of the skin for low PG dose studies and hence the 2 tape strips were only used in this arm of the study). The cotton tip of the cotton buds and the two tape strips from each cell (if used) were then immersed, separately, into 4 mL of 20% v/v ethanol in PBS (pH 7.2). The epidermal sheet was then placed into 5 ml acetonitrile. All the samples were incubated overnight at 32˚C (shaking water bath at 120 strokes.min^{-1}), after which the solvent was filtered using 0.2 μm poly(vinylidene fluoride) syringe filters (Whatman®, New Jersey) and analysed for diclofenac content using HPLC. All the extraction methods were

proven 'fit for purpose' as per OECD Guideline for the Testing of Chemicals (2004) [24]. The total % recovery for the mass balance process was 95.35 ± 5.52% (Dic, *n* =4) and 93.75 ± 2.21% (InDicP, *n* =4) for the high PG loading dose and was 89.9 ± 4.7 % (InDicP, *n* = 5) for the low PG loading dose. All the skin permeation experiments were repeated 4-7 times. The chemical stability of diclofenac in spent receiver fluid (PBS, 0.172 M, pH 7.4) was assessed at three diclofenac concentrations (8.5, 45 and 85 $\mu g.mL^{-1}$) over 24 h. The average % recovery from the procedure was 101.7 ± 1.77 % (*n* = 15), which indicated the chemical stability of the drug was not influence by the skin tissue matrix.

Diclofenac and propylene glycol quantification

Quantitative determination of diclofenac in the transport studies was performed using a reverse phase HPLC system consisting of a Jasco UV detector and pump (Jasco Corporation Ltd, UK). The mobile phase comprised acetonitrile:methanol:formate buffer (25 mM) (50:20:30 (v/v), pH 3.5) set at a flow rate of 1.2 $mL.min^{-1}$. Diclofenac was eluted from a Gemini® C18 (250 × 4.6 mm) stationary phase (Phenomenex, UK) at room temperature using a 20 μL injection volume and UV detection at 275 nm. The retention time for diclofenac was 7.5 min and the assay was previously shown to be 'fit for purpose' [12]. The calibration curves were constructed on the basis of the peak area measurements using standard solutions of known diclofenac concentrations dissolved in an identical fluid as the receiver phase for the permeation studies and an identical fluid as extraction fluid for the recovery studies.

Quantitative determination of PG in the receiver aliquots sampled over the 26-h time period of the skin distribution studies was determined indirectly by assessing the increase in

diclofenac saturated solubility due to the amount of PG permeating to the receiver fluid, using a PG-solubility calibration curve (method modified from Bendas et al., [8]). Calibration solutions containing PG in the range of 0 to 41 $mg.mL^{-1}$ (i.e. 0 to 4% v/v PG) in spent receiver fluid were saturated with diclofenac (as described previously for the formulation preparation) and the saturated solubility of diclofenac at each PG concentration was determined using HPLC. The PG-solubility calibration curved showed a good linearity (R^2 = 0.96). The assay LOD was 8.1 $mg.mL^{-1}$, LOQ was 24.6 $mg.mL^{-1}$ and the accuracy was determined to be 99.6 ± 10.5 % (n = 8).

Data analysis

Cumulative amounts of drug (μg) penetrating per unit surface area of the ES (cm^2) were corrected for recovery sample removal and plotted against time (h). The steady-state transmembrane transport rate, flux, was taken from the line of best fit over at least 4 time points with values above the limit of detection (LOD) of the HPLC assay, and with a linearity of $R^2 \geqslant 0.98$. The lag time (T_L) was obtained by extrapolation of the steady-state gradient from each individual Franz cell experiment. The enhancement ratio (ER) was defined as the ratio of steady-state flux when applied from InDicP formulation to that produced when applied as the saturated control Dic. As two pieces of skin were used for different PG loading (necessitated by the time frame of the studies and the size of the donated skin samples) when the data were compared from the two different skin donors a ratio of epidermal to dermal skin deposition was employed to minimised the influence of donor to donor variability.

Cumulative amounts of PG (mg) penetrating per unit surface area of the ES (cm^2) were corrected for previous sample removal and plotted against time (h). Steady-state flux was

taken from the line of best fit over at least 4 time points with values above the limit of detection (LOD) of the PG-solubility assay, and with a linearity of $R^2 \geq 0.96$. All values were expressed as their mean ± standard deviation (SD), and statistical analysis of data was performed using the statistical package for social sciences SPSS version 16.0 (SPSS Inc., Chicago, IL. USA). Normality (Sapiro-Wilk) and homogeneity of variances (Levene's test) of the data were assessed prior to statistical analysis. Permeation results were analysed statistically using one way analysis of variance (ANOVA) tests with post-hoc Tukey analysis where required. All other data were analysed using a Student's *t*-test. Statistically significant differences were assumed when $p \leq 0.05$.

The theoretical amount of solvents in the skin (*sc* and epidermis) was calculated on the basis of a literature reported steady-state flux of water at 0.2 $mg.cm^{-2}.h^{-1}$ [25-26] and a measured steady-state flux of at PG at 9 $mg.cm^{-2}.h^{-1}$ (using PG at 240 $\mu g.cm^{-2}$) and 1 $mg.cm^{-2}.h^{-1}$ (using PG at 2.2 $\mu g.cm^{-2}$). These penetration rate values were expressed as a 3-D penetration rate ($mg.cm^{-3}.h^{-1}$) by taking into account the skin strata thicknesses. Using the solvent's densities, the volume of PG and water penetrating the *sc* and epidermis was then calculated at 1 h post-dose application, as described in equations 1 and 2.

$$vH_2O_{t,sc} = \left(\frac{Flux_{H_2O} / h_{sc}}{D_{H_2O}} \right) \times t \qquad (1)$$

$$vPG_{t,sc} = \left(\frac{Flux_{PG} / h_{sc}}{D_{PG}} \right) \times t \qquad (2)$$

Where $vH_2O_{t,sc}$ and $vPG_{t,sc}$ (mL) were the volumes of water and PG penetrating the *sc* at time *t* (1 h), $Flux_{H2O}$ and $Flux_{PG}$ were the steady-state flux values of water and PG ($mg.cm^{-2}.h^{-1}$), h_{sc} the *sc* thickness (at 15×10^{-4} cm) and D_{H20} and D_{PG} were the density values for water and PG ($mg.ml^{-1}$), respectively. The volumes of water and PG penetrating the epidermis at time *t* were determined in a similar manner by using the thickness of the epidermis, h_{ep} (at 100×10^{-4} cm). The volume of endogenous water in each layer ($vH_2O_{endog,\ sc}$ estimated at 8.3×10^{-4} mL (*sc* water content = 20% w/w, wet density = 1.39 $g.cm^{-3}$ [27]) and $vH_2O_{endog,\ ep}$ estimated at 5.1×10^{-3} mL (epidermis water content = 60% w/w, wet density = 0.43 $g.cm^{-3}$ (calculated from the reported dry density at 0.17 $g.cm^{-3}$ [28])) was added to the exogenous penetrating water in order to determine the v/v ratio of PG to water in the *sc* and the epidermis at 1 h (equations 3 and 4):

$$\%vH_2O_{t,sc} = \left(\frac{vH_2O_{t,sc} + vH_2O_{endog.}}{vH_2O_{t,sc} + vH_2O_{endog.} + vPG_{t,sc}} \right) \times 100 \qquad (3)$$

$$\%vPG_{t,sc} = \left(\frac{vPG_{t,sc}}{vH_2O_{t,sc} + vH_2O_{endog.} + vPG_{t,sc}} \right) \times 100 \qquad (4)$$

The current model assumed that there was no significant displacement of water molecules by PG and that the solvent's penetration rate across the *sc* and the epidermis was homogeneous.

Results

Formulation characterisation

Analysis of the diclofenac formulation vehicle showed that the addition of water to PG at a volume ratio of 50:50 induced redshifts in the C-O stretching, CH stretching and OH deformation of PG (Fig SI 1a). These shifts confirmed the formation of PG-rich supramolecular complexes and a consequential reduction in water structuring in the co-solvent system [12]. This was further supported by the 4 cm^{-1} down-field shift in the bending vibration of pure water (D_2O) when compared to the 50:50 PG:water solution (Fig SI 1b). The infrared spectra of the PG-water co-solvent was unchanged when the drug and polymer were added to the system and similarly the infrared spectra were unaffected by the *in situ* self-assembly process of the HPMC microparticles when the two spray solutions were mixed (i.e. mimicking the application of the InDicP formulation to the skin) (Fig.SI 1).

Mixing PG and water without HPMC generated crystals with a different morphology compared to the diclofenac microparticles (Fig 1). The birefringence of both materials, when exposed to cross-polarised light, confirmed that they were crystalline in nature and this result suggested that the InDicP microparticles consisted of a diclofenac crystalline core in a HPMC shell. This finding accorded with the analysis of a similar system formed from a 70:30 PG-water solvent mixture [20].

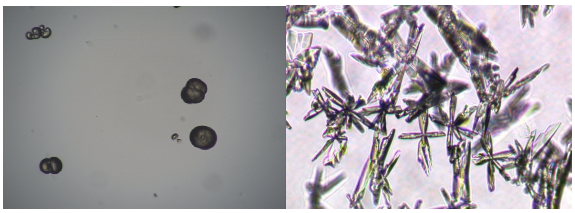


Figure 1. Diclofenac crystal morphology generated in the diclofenac supersaturated microparticle suspension (InDicP) (left) and that obtained in an equivalent control system without the polymer hydroxymethylcellulose (right). The magnification used was × 200.

Diclofenac permeated across silicone membrane rapidly with no transport lag-time when the InDicP was used to deliver the drug. Within the first 6 h, no donor phase dose depletion or receiver phase saturation occurred, and hence steady-state flux was measured in this time period ($R^2 > 0.99$). Diclofenac steady-state flux, when applied as the InDicP formulation (at 24.7 ± 6.4 $\mu g.cm^{-2}.h^{-1}$, n = 5 ± SD) was significantly higher ($p < 0.05$) than that obtained from a drug-saturated control (at 1.69 ± 0.13 $\mu g.cm^{-2}.h^{-1}$, n = 5 ± SD) (Supplementary information SI2). Using the calibration curve (Fig. 2 inset, data from Benaouda *et al.,* [22]), diclofenac DS in the solution state for InDicP during steady-state transport was calculated to be 15.6 ± 4.1.

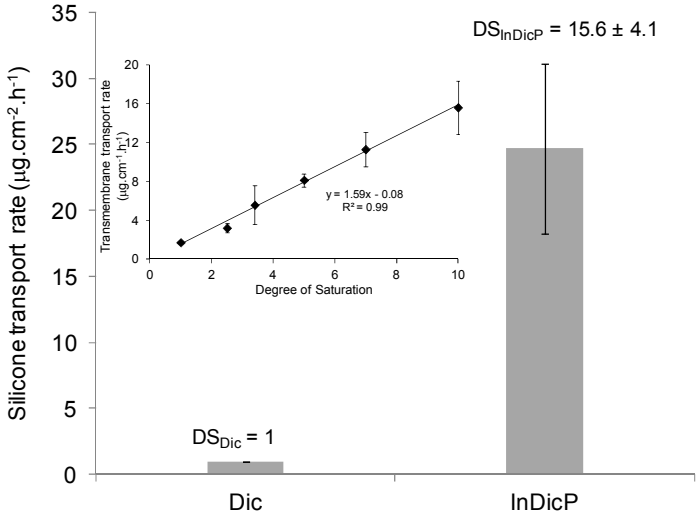


Figure 2. Diclofenac degree of saturation in the diclofenac supersaturated microparticle suspension (InDicP) and the saturated control (Dic) (n =5 ± SD). The inset represents diclofenac transport calibration curve using a silicone membrane (n = 5 ± SD) (data from Benaouda *et al*., [22]).

Human skin studies

PG permeated across the human ES to a similar extent ($p > 0.05$) when the InDicP and Dic formulations were used to apply a 240 µg.cm^{-2} dose of PG to the apical surface of the tissue (Figure 3). The steady-state flux of PG, at ca. 9 mg.cm^{-2}.h^{-1}, was recorded between 4-8 h, post-dose application. At 8 h post-dose application, the PG steady-state permeation was lost, since 23.3 ± 5.0 % and 37.5 ± 8.9 % of the applied PG using Dic and InDicP formulations, respectively, had permeated from the donor solutions. The PG flux reduced to ca. 1 µg.cm^{-2}.h^{-1} after the 8 h time point and it remained at this rate for the subsequent 12 h.

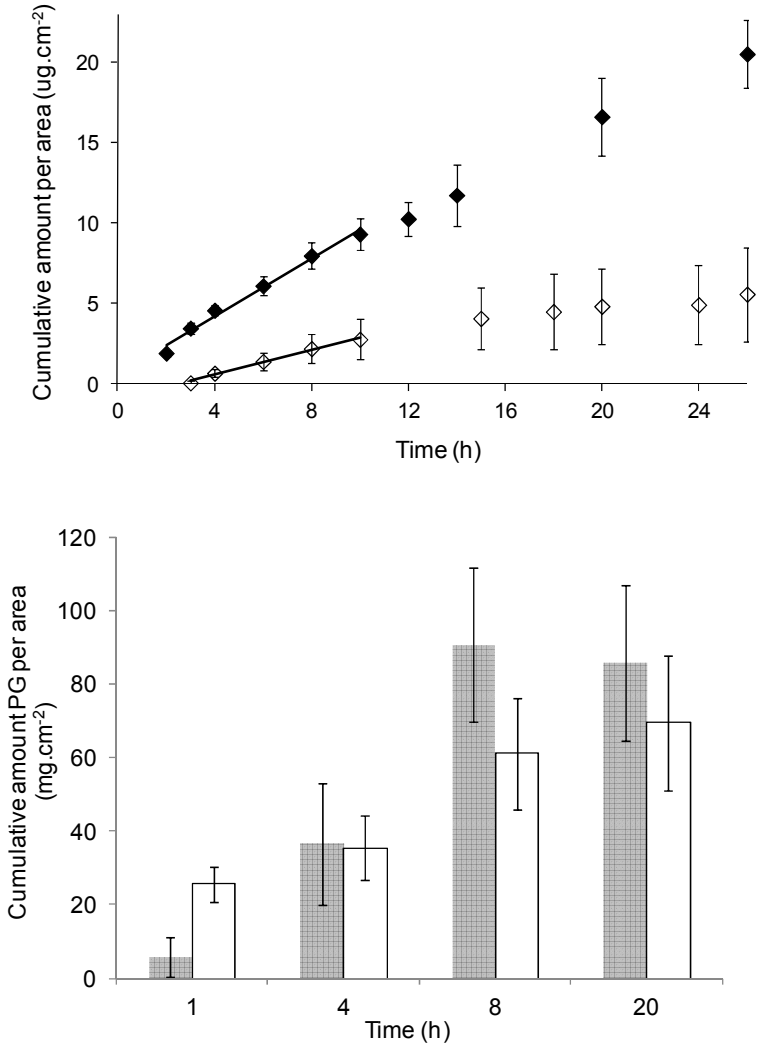
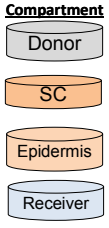


Figure 3. a) Diclofenac transport across human epidermal sheet when presented as drug-saturated control (Dic, ◊), and as the diclofenac supersaturated microparticle suspension (InDicP, ♦). b) The cumulative mass of propylene glycol permeated using Dic (open bars) and InDicP (grey bars) formulations (n = 5-7 ± SD).

The 1 h post dose time point was chosen to compare the co-solvent ratios in the skin as this was thought to provide the most accurate 'snap shot' of what was occurring in terms of supramolecular structuring in the tissue during the steady state permeation process. At this 1

h post dosing time point the *in silico* calculations predicted that the application of the 240 μg.cm^2 of PG would produce 4.2 × 10^{-3} ml of PG per mg of *sc* and 2.0 × 10^{-3} ml of PG per mg of epidermis. The water content would be expected to be 9.5 × 10^{-5} ml per mg of *sc* and 4.7 × 10^{-5} ml per mg of epidermis. From these values the PG-water (v/v) ratio was calculated to be 93:7 and 76:24 in the *sc* and epidermis, respectively (Fig. 4). Previous studies have shown that PG-rich supramolecular structures predominate at both these solvent ratios [12].



Compartment	High PG loading dose (PG at 0.24 mg.cm^{-2}) PG:H_2O (v/v)	Solvent Suprastructure	Dic-Solvent Interaction	Low PG loading dose (PG at 2.2 × 10^{-3} mg.cm^{-2}) PG:H_2O (v/v)	Solvent Suprastructure	Dic-Solvent Interaction
Donor	50:50	PG-rich and H_2O-rich	Moderate	50:50	PG-rich and H_2O-rich	Moderate
SC	93:07	PG-rich	Good (High solvency capacity)	38:62	H_2O-rich	Weak (Low solvency capacity)
Epidermis	76:24	PG-rich	Good (High solvency capacity)	10:90	H_2O-rich	Weak (Low solvency capacity)
Receiver	0:100	H_2O structuring	Weak	0:100	H_2O structuring	Weak

Figure 4. Propylene glycol/water volume ratio changes upon solvent entry into the *stratum corneum* (SC) and epidermis at 1 h after the application of the diclofenac supersaturated microparticle suspensions using a high and a low PG loading dose.

When the 240 μg.cm^{-2} dose of PG was used to apply the diclofenac to the apical surface of the skin its permeation across human ES was significantly faster ($p < 0.05$) compared to the drug-saturated control (the flux ca. 3.4-fold higher and the lag time ca. 3.5-fold lower) (Fig. 3, Table 1). It was noted that 10 h after the application of the formulations the steady state permeation of the drugs seemed to cease and the drug flux slowed. At this time point both the drug-saturated control formulation, Dic, and the InDicP had delivered < 10% of the applied dose (at 7.1 ± 2.9 % (Dic) and 1.4 ± 0.1 % (InDicP)), hence drug depletion did not seem to drive the loss in steady-state observed in the transport profile (Fig. 3). As the loss of

diclofenac steady-state permeation occurred at the same time that PG depleted in the donor phase the change in flux was attributed to the associated change in PG transport into the skin.

Table 1. Diclofenac and propylene glycol (PG) permeation parameters across human epidermal sheet (ES) following the application of diclofenac saturated control solution (Dic, $n = 4 \pm$ SD) or the diclofenac supersaturated suspension (InDicP, $n = 7 \pm$ SD) using PG at a high loading dose. The parameters are: steady-state transmembrane rate (flux), lag time (T_L), diclofenac mass retained in the epidermis at 26 h ($Q_{epider., 26\ h}$), the cumulative diclofenac mass permeated across the skin at 26 h ($Q_{transder., 26\ h}$), the cumulative PG amount permeated at 8 h ($Q_{transder,8h}$), expressed as a fraction (%) of the applied PG, and the enhancement ratio (ER) compared to the control.

	Diclofenac				Propylene glycol	
	Flux ($\mu g.cm^{-2}.h^{-1}$)	T_L (min)	$Q_{epider., 26\ h}$ ($\mu g.cm^{-2}$)	$Q_{transder., 26\ h}$ ($\mu g.cm^{-2}$)	Flux ($mg.cm^{-2}.h^{-1}$)	$Q_{transder, 8h}$ (%)
Dic	0.4 ± 0.2	115.2 ± 21.2	138.7 ± 11.7	20.5 ± 2.1	**	23.3 ± 5.0
InDicP	1.1 ± 0.2*	35.5 ± 10.7*	0.8 ± 0.1	5.6 ± 2.9	9.3 ± 4.4a	37.5 ± 8.9
ER	3.4 ± 1.2	3.5 ± 1.2	174.3± 21.5	4.4 ± 1.6	-	1.7 ± 0.5

*indicates statistical difference compared to the control (t-test, $p < 0.01$).
** values below the assay's LOD
[a] n = 3

At 26 h post-dose application, for InDicP, the epidermal distribution of the diclofenac (at 138.6 ± 11.7 $\mu g.cm^{-2}$) was 174.3 ± 21.5 times higher ($p < 0.05$) than when the saturated control solution, Dic, was used to apply the drug to the apical surface of the skin (at 0.8 ± 0.1 $\mu g.cm^{-2}$) and this represented 22% (InDicP) and 2% (Dic) of the diclofenac available in the donor in the solution state. At this time point, the transdermal permeation of the drug was 4.3 ± 0.2 times higher ($p < 0.05$) for the InDicP compared to the Dic system (Table 1). The epidermal/transdermal distribution ratio of diclofenac was shown to be 6.8:1 and 0.1:1, 26 h after the dose application of InDicP and Dic, respectively.

Reducing the PG loading dose to 2.2 $\mu g.cm^{-2}$ produced an epidermal/transdermal distribution ratio of diclofenac of 0.7:1 for the InDicP system (Fig. 5) (epidermal diclofenac at 2.5 ± 0.9 $\mu g.cm^{-2}$ and transdermal diclofenac at 3.7± 1.9 $\mu g.cm^{-2}$). On the basis of the PG steady-state measured in the previous permeation studies (at 9 $mg.cm^{-2}.h^{-1}$), it was anticipated that PG would rapidly deplete from the donor phase, and hence a PG flux of 1 $\mu g.cm^{-2}.h^{-1}$ was used to calculate the solvent skin distribution at 1 h after the formulation was applied to the skin. Using this PG flux value at the 1 h time point for the 2.2 $\mu g.cm^{-2}$ PG loading the PG skin deposition was calculated to be 1.4×10^{-4} ml per mg of *sc* and 6.8×10^{-5} ml per mg of epidermis, whereas the water content was 2.8×10^{-5} ml per mg of *sc* and 1.4×10^{-5} ml per mg of epidermis. These values gave a PG-water (v/v) ratio of 38:62 in the *sc* and 10:90 in the epidermis 1 h after dose application (Fig. 4). Infrared spectroscopy analysis conducted previously *in vitro* suggested that at these v/v ratios water-rich solvent supramolecular structures prevail in the epidermal tissue [12].

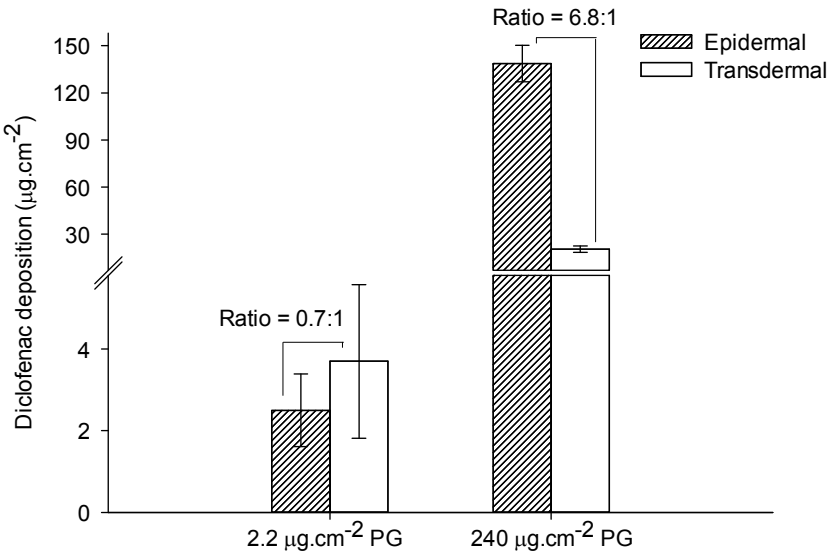


Figure 5. Diclofenac epidermal and transdermal disposition at 24 h post-application of diclofenac supersaturated microparticle suspension using a high (grey bars) and a low (open bars) loading dose of propylene glycol (n = 5 ± SD).

Discussion

The PG:water matrix deposited on the surface of the skin by the two pump sprays supported a high diclofenac thermodynamic activity (DS ca. 16) despite the formation of the solid phase. These results were in accordance with previous work that generated a similar diclofenac microparticle suspension using a 70:30 (v/v) PG:water matrix [20]. As described in previous work the microparticles were spherical in shape; they displayed a uniform size distribution and contained a crystalline core [20]. Therefore, the microparticle suspensions were thought to act in a similar manner as the diclofenac suspensions employed for the permeation studies, whereby the solid phase acted as a reservoir that replenished the soluble diclofenac in order to sustain a high diclofenac thermodynamic activity [20].

The supersaturation of diclofenac in the formulation that occurred when the InDicP system was dosed to the apical surface of the skin provided the thermodynamic drive to allow the drug to move into the tissue rapidly. The FTIR analysis suggested that the 50:50 PG:water co-solvent ratio supported this process through the disruption of the water structuring in the vehicle, which increased the availability of hydrogen bond acceptors in the drug supersaturated solution. However, using a high PG loading dose generated an epidermal deposition of diclofenac that was 175-times higher than the saturated control and this exceeded the 16-fold increase that was expected from the drug supersaturated vehicle with a thermodynamic activity of 16 (a direct correlation between DS and flux with these systems has been shown in homogeneous membranes [20, 22]). This suggested that the increase in the thermodynamic activity of the drug when it was applied to the skin was only in part responsible for the enhanced epidermal deposition showed by the spray formulation when compared to a drug-saturated control.

The relationship between the loss of diclofenac and PG steady-state flux into the skin provided support to the notion that the solvent supramolecular structuring was influential in the transfer of the drug from the point of administration, on the apical surface of the skin, into the tissue. The significance of co-localising PG within skin tissue to the percutaneous penetration of several drugs has been previously discussed in the literature [7, 9, 29]. For example, Trottet et al., reported that the depletion of PG from an applied formulation reduced the percutaneous transport of loperamide [29], while, Squillante et al., using ^{14}C-PG, reported the concomitant percutaneous diffusion of PG and nifedipine [9]. However, this previous work did not consider the significance of the rapid permeation of PG into the skin on the PG:water ratio within the tissue. The calculations performed in this work showed that the rate of PG entry into the skin produced a situation that allowed the excipient, at certain time points of

the permeation experiments, to dominate the interactions in the tissue. One consequence of the high PG levels in the skin tissue was that it would disrupt the normal drug-water and drug-tissue interactions due to its preferential association with the drug [20].

The changes in the epidermal/transdermal distribution ratio of diclofenac after 24 h compared to the drug-saturated control suggested that the PG water co-solvent was promoting diclofenac penetration particularly into the epidermal tissue. PG has previously been found to localise in the skin at PG:water ratios similar to those calculated in this work; for example, the PG-water ratio was found to be 80:20 (v/v) in human *sc* following a 24 exposure to a 60:40 PG-water mixture [10]). However, this previous work did not consider the potential to have different PG water ratios in the different skin strata and hence they did not determine the effect of this phenomenon on drug deposition in the skin. It was evident from the data gathered from present study that PG also had a particular skin deposition profile after topical application and that this profile could be altered through the application of different quantities of the excipient. High loading doses of PG applied to the skin produced high levels in the epidermis and this enabled diclofenac to preferentially reside in this skin strata. When the PG application dose was reduced by 100-fold, there was a marked change in the solvent skin distribution which was brought about by the rapid depletion of PG from the donor phase. A low level of PG in the skin reduced the PG in the sc and epidermis to such an extent that water became the predominant solvent in these layers of tissue (PG-water ratios at 40:60 (sc) and 10:90 (epidermis)). The consequence of water being the predominant solvent in the epidermis was that the preferential drug deposition was eliminated.

Attempts to try and explain the diclofenac's preferential residence in epidermis when accompanied by high levels of PG using Fedor's solvency calculations did not improve the

understanding of the system [30]. Calculating the solubility parameter of the skin (δ = 9.7-10 $(cal.cm^{-3})^{1/2}$ [31]), diclofenac (δ = 10.86 $(cal.cm^{-3})^{1/2}$) and the potential changes in the solubility parameter of the skin upon the use of the high PG dose ($\delta_{PG\text{-}water,\ epidermal}$ = 16.5 $(cal.cm^{-3})^{1/2}$) and low PG dose ($\delta_{PG\text{-}water,\ epidermal}$ = 22.5 $(cal.cm^{-3})^{1/2}$) suggested that the PG rich solvent system should have very little benefit in localising diclofenac in the skin. However, Fedors method is based on the principles of ideal mixing, which does not apply to the PG-water solvent system. The inability of Fedor's solvency calculations to explain the effects of the solvents indicates that the non-ideal mixing of the PG:water co-solvents was important in the manner in which the PG aided penetration of diclofenac into the skin.

The direct FTIR analysis of the PG and water co-solvent interactions in the skin was not possible due to the overlapping of the spectra signals that provided the basis for the solvent-solvent associations. Therefore, the interactions of the drug with the solvent at the ratios calculated to be in the skin at any given time point were based upon the previous FTIR analysis of binary PG-water mixtures at similar solvent volume ratios performed *in vitro* [12]. It is accepted that the calculations of the solvent ratios in the skin may have contained inaccuracies due to the sourcing of reference values from different studies. However it was thought that these inaccuracies would not have a marked effect on the outcomes reported in this work, since the calculated values were unlikely to be altered by more than 10%. Such a value was predicted to be the critical value necessary to alter any solvent supramolecular structuring markedly. The *in vitro* FTIR data suggested that the predominance of PG-rich supramolecular complexes, accompanied with the loss of water structuring, would hold unionised diclofenac molecules in a drug solvent complex through physical interactions [12]. In the current study, the PG-rich solvent complexes formed within the skin would be anticipated to display similar properties and hence this was used as the basis upon which to explain the

epidermal localisation of the diclofenac molecules when PG was the dominant solvent in the skin strata. When the PG loading in the application vehicle was reduced, water-rich solvent supramolecular structures were thought to be formed. These water rich structures have been shown to have a limited capacity to interact with the diclofenac molecules and hence such structures would be unlikely to retain diclofenac molecules within the skin to the same degree as when the tissue contains a high level of PG [12].

Conclusion

The epidermal localisation of diclofenac was thought to be facilitated in this study through the formation of PG rich supramolecular solvent structures within the skin. This effect was reversed when the PG levels dropped in the epidermis. FTIR data suggested that when only a small loading dose of PG was used to administer the diclofenac to the skin water rich supramolecular solvent structures dominated in the epidermis and such structures had a lower capacity to bind to diclofenac and retain the drug in the tissue. These findings require *in vivo*, confirmation but if similar effects are seen in man they would provide a sound basis for the design novel topical dosage forms that can control active agent delivery using supramolecular solvent complexes. These results clearly demonstrate that the formulation of topical vehicles is an integral facet of modulating the delivery of pharmaceutical compounds to the skin.

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